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## RESEARCH ARTICLE

# Factors influencing the erosion rate and the drug release kinetics from organogels designed as matrices for oral controlled release of a hydrophobic drug

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### Abstract

This article proposes solid-like systems from sunflower oil structured with a fibrillar network built by the assembly of 12-hydroxystearic acid (12-HSA), a gelator molecule for an oil phase. The resulting organogels were studied as oral controlled release formulations for a lipophilic drug, Efavirenz (EFV), dissolved in the oil. The effects of the gelator concentration on the thermal properties of the organogels were studied by Differential Scanning Calorimetry (DSC) and showed that drug incorporation did not change the sol-gel-sol transitions. The erosion and drug release kinetics from organogels under conventional (filling gelatin capsules) or multiparticulate (beads obtained by prilling) dosage forms were measured in simulated gastric and intestinal fluids. EFV release profiles were analyzed using model-dependent (curve-fitting) and independent approaches (Dissolution Efficiency DE). Korsmeyer-Peppas was the best fitting release kinetic model based on the goodness of fit, revealing a release mechanism from organogels loaded with EFV different from the simple drug diffusion release mechanism obtained from oily formulations. From organogels, EFV probably diffuses through an outer gel layer that erodes releasing oil droplets containing dissolved EFV into the aqueous medium.

### Keywords

Efavirenz, lipid formulation, multiparticulate dosage form, oral drug delivery, organogel, poorly soluble drugs, prilling process, release profile models

### Introduction

Interest in lipid-based drug delivery has developed over the past 15 years, largely driven by the growing need for novel drug delivery systems to deal with the vast majority of the new chemical entities (NCE) that have poor solubility, or permeability or to improve the delivery of existing drugs<sup>1</sup>.

The diversity of commercialized lipid excipients (e.g. fatty acids, glycerides, polyoxyethylene glycols derivatives, ethoxylated glycerides and polyalcohol fatty acid esters) has created a large platform for lipid-based formulation design. Depending on the choice of lipid excipients and formulation techniques, it is possible to obtain a variety of systems in the forms of emulsions, microemulsions, micelles, liquid crystalline particles, solid lipid particles and various self-emulsifying systems.

Successfully marketed oral lipid-based drug products, currently concerning 54% of the global pharmaceutical market, are mostly formulated as bulk liquid solutions or liquid-filled gelatin capsules<sup>2</sup>. Clinical translation of novel lipid-based formulations is clearly related to the rate of discovery of pipeline drugs exhibiting poor aqueous solubility, which is predicted to increase from 30%

to 70%<sup>3</sup>. Major barriers for attaining full commercial potential for lipid-based formulations are associated with challenges in their physical stability, non-ideal drug encapsulation and unpredictable delivery performance.

The primary mechanism of action by which a lipid formulation leads to improved bioavailability is usually avoidance of the slow dissolution process that limits the bioavailability of hydrophobic drugs from solid dosage forms. A formulation keeping the drug in a dissolved state throughout its transit in the gastrointestinal tract (GI tract) appears preferable. In this context, a gel vehicle and especially an organogel could be useful to improve the bioavailability of a hydrophobic drug. In general, a gel may be defined as a semi-solid formulation having an external solvent phase, apolar (organogel) or polar (hydrogel) immobilized by a 3D-network scaffold. Organogels may be regarded as bi-continuous systems consisting of a gelator and an apolar solvent. The gelators may undergo physical or chemical interactions so as to form self-assembled entangled fibrous structures leading to the formation of a three-dimensional scaffold. The 3D-networked structure, hence formed, entraps and immobilizes the apolar phase.

In the last 20 years, there has been an explosive growth in research on organogels, and on publications related to them<sup>4-11</sup> as another category of lipid system. Most of these publications report the discovery and/or the synthesis of new organogelators, the properties of organogels including the gel microstructures and the manner in which the gelator molecules could be arranged

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in 3-D network structures. The large diversity in terms of the possible microscopic structures available for organogels originated the interest for applications of these soft materials in the petrochemical industry for gelling flammable solvents for storage and transport<sup>12</sup>, in the food industry for structuring edible oils<sup>13</sup>, in the cosmetic field<sup>14</sup> and more recently as part of pharmaceutical drug delivery systems. As far as drug delivery is concerned, organogels appear as suitable carriers for lipophilic drugs. Consequently, in recent years, the possibility of using organogels as drug delivery vehicles has been explored for parenteral<sup>9</sup> and transdermal delivery<sup>8,10</sup>. Nevertheless, so far, only few studies have been carried out on oral drug delivery<sup>15,17</sup>.

Starting from these considerations, this study proposes the application of organogels to integrate attractive and novel lipid-based delivery systems for controlled release of a hydrophobic drug, Efavirenz (EFV), via oral administration. EFV is a non-nucleoside reverse transcriptase inhibitor, used for the treatment of the human immunodeficiency virus (HIV). EFV is a molecule of class II of the biopharmaceutical class system<sup>18</sup> that presents low aqueous solubility (4 µg/ml)<sup>19</sup> and high permeability. Efavirenz (EFV) is a first-line anti-HIV drug largely used as a non-nucleoside reverse transcriptase inhibitor as part of antiretroviral therapies. EFV as active pharmaceutical ingredient (API) is commercially available as micronized powder. Some papers<sup>20,22</sup> have been published focusing on EFV innovative solid forms, but there is no clear evidence of solubility or dissolution improvement. Other studies<sup>23,27</sup> have led to an amorphous system that was inherently unstable, could recrystallize and had low physical stability. Organogels for oral administration of EFV could represent an innovative drug delivery system, particularly under multiparticulate forms.

Among the different physical methods available to obtain multiparticulate forms, such as spray drying, fluid bed coating or extrusion, prilling is able to produce microparticles or beads (prills) with very narrow dimensional range and high encapsulation efficiency. These beads are produced by breaking apart a laminar jet of molten organogel into a row of monosized drops by means of a vibrating nozzle device. The aim of this study was also to show that prilling technique could be used as a simple method to prepare organogel beads with narrow particle size distribution loaded with Efavirenz, as multiparticulate dosage forms. Multiparticulate dosage forms are known to overcome the poor and variable GI tract absorption of drugs and have shown the ability to reduce or eliminate the influence of food on bioavailability<sup>28</sup>.

In view of the above, the control release of EFV from sunflower oil-based organogel structured with 12-hydroxystearic acid (12-HSA) as gelator has been studied. The 12-HSA was chosen because it is one of the most studied molecular gelators for cosmetics<sup>29</sup> and more recently for food<sup>17</sup> and drug delivery applications<sup>15</sup> in part due to its inexpensive cost, versatility in gelling numerous solvents<sup>30</sup> and safety<sup>29</sup>. The effect of different parameters (gelator concentration, temperature of the gel formation, EFV loading, composition of the release media) on the thermal properties of the gels and drug release kinetics was investigated. The erosion rate was also investigated for the gel capsules.

## Materials and methods

### Materials

A food grade sunflower oil (density of 0.92 g/mL) provided by a local supplier was chosen as the vegetable oil for organogel preparation. Sunflower oil is mainly composed of linoleic acid (60–703 w/w), but it also contains oleic acid (14–353 w/w), palmitic acid (3–103 w/w) and stearic acid (1–103 w/w)<sup>31</sup>.

12-Hydroxystearic acid (12-HSA 853, C<sub>18</sub>H<sub>36</sub>O<sub>3</sub>, MW 300.48 g/mol) was purchased from Alfa Aesar (France) and used as gelling agent. Efavirenz (EFV) was obtained from Cristalia S.A. (Itapira, Brazil). Acetonitrile used in solubility, pepsin and pancreatin used in the erosion/drug release studies were supplied by Sigma-Aldrich (France). Sodium dodecyl sulfate (SDS) was supplied by VWR (Prolabo, France).

### Methods

#### *Drug solubility in gel and oil phases*

*EFV solubility in molten organogels.* The enthalpies of solution of EFV in molten organogels were carried out isothermally (90.0 ± 0.5 °C) using a heat flux in a Calvet calorimeter model-C80 (Setaram, France) equipped with two mixture cells, one for reference. Each cell has an upper and lower compartment separated by a displaceable lid that allows the connection of compartments during the analysis. The sample cell was filled with accurately weighed amount of drug (30, 60, 70, 75 and 80 mg EFV/g organogel) in the lower compartment and 1 g of organogel in the upper compartment. After thermal stabilization, the vessel was rotated by 180° several times, which displaces the lid between the drug and solution leading to their mixing. The signal was automatically recorded on the strip chart recorder. Experiments were conducted in duplicate.

*EFV solubility in sunflower oil.* The solubility of Efavirenz in sunflower oil was determined at 37 ± 1 °C by adding excess drug (5 g) to the oil (50 g). The suspensions were equilibrated in a constant temperature shaking water bath during 24, 48 and 72 h. An aliquot of the filtered samples (1 mL) was transferred to a volumetric flask for appropriate dilution with acetonitrile (ACN); subsequently, 1 mL of the resultant solution was diluted with the mobile phase acetonitrile: acetate buffer (60:40) and analyzed by High Performance Liquid Chromatography (HPLC) (LaChrom Elite, Hitachi) to determine the amount of dissolved EFV. The HPLC was equipped with reversed-phase column C18, 250 x 4.6 mm, 5 µm (Kinetex™ - Phenomenex, France) and the HPLC column operating conditions were optimized [mobile phase ACN: acetate buffer (60:40), 1.0 mL/min flow rate, volume injection of 40 µL, UV-detector 252 nm]. Experiments were conducted in triplicate.

#### *Thermal analysis*

12-HSA was added to sunflower oil over a range of compositions (2–303 w/w). Samples were prepared by dissolving the weighed solid organogelator in a weighed amount of liquid oil at 85 °C under stirring (300 rpm). The heated solutions were subsequently cooled to room temperature (25 °C) and stored at this temperature for further analysis. For the formulations including EFV in the organogel (30 or 60 mg EFV/g organogel), both EFV and 12-HSA were weighed and added to sunflower oil. The mixture was then heated at 85 °C with gentle stirring during 30 min to melt the organogel and dissolve EFV. The heated solutions were subsequently cooled to room temperature and stored under the same conditions for further analysis. AU concentrations are expressed as a weight percentage (wt%).

Thermal properties of organogels, unloaded or loaded with EFV, were determined by Differential Scanning Calorimetry (DSC) using a FRS5 calorimeter (Mettler Toledo, France). Samples (5–10 mg) were hermetically sealed in aluminum standard pans. Temperature was heated and cooled at a rate of 3 °C/min from 15 to 85 °C for two cycles. Before the second cycle, the system was kept at 15 °C for 20 min. A nitrogen gas purge was used at 20 mL/min. Melting (T<sub>m</sub>) and crystallization

(T<sub>c</sub>) points were determined from DSC data (onset, peak and end temperatures as well as the associated specific enthalpies).

#### *Erosion and release kinetics from organogel in a conventional dosage form (cylindrical shape)*

The erosion mode of the delivery system is one of the factors controlling drug release. In order to understand the release kinetics in relation to their weight changes, both erosion rate and release rate were determined and correlated.

Molten organogels loaded with EFV were solidified in hard shell gelatin capsules (size #00: i.e. 0.95 mL of capacity volume; length of 23.3mm and diameter of 8.2mm) at 25 or 5 °C. The dissolution of gelatin capsules after five minutes of contact with the dissolution media did not affect erosion nor release rate. Two gelator concentrations were used (5 and 20 wt% of 12-HSA). Erosion and drug release were measured from the organogels in a SOTAX AT7 (Switzerland) dissolution bath at 37 ± 0.5 °C. The organogels capsules were weighed, placed in a USP dissolution basket and immersed in 500 mL dissolution media at 50rpm. The simulated gastric solution comprised pepsin (800 U/L of enzyme activity) dissolved in 0.1 N hydrochloric acid (pH 1.2) and the simulated intestinal solution included pancreatin (1750 U/L of protease activity and 140 U/L of lipase activity)<sup>32</sup> dissolved in phosphate-buffered saline (pH 6.8). Both solutions contained 1.0wt% SDS according to dissolution monograph of Efavirenz. The organogels were removed from the test solution (1, 2, 3, 4, 6 and 8 h after starting) and dried under ambient conditions during 24 h. Determinations were performed in duplicate.

Organogel erosion was determined by difference in weight, taking the amount of the drug release into account. Percentage of organogel erosion (E) at time *t* was calculated from Equation (1).

$$E(\%) = \frac{(m_0 - m_t - m_{EFV\text{released}})}{m_0} \times 100 \quad (1)$$

Where *m*<sub>0</sub> is the initial weight of the organogel, *m*<sub>*t*</sub> the weight of the organogel subjected to erosion for time *t* and *m*<sub>EFVreleased</sub> the weight of EFV released at time *t*.

When the organogel was removed from the test solution, 5 mL of the medium was also withdrawn from the incubation vessel. The amounts of EFV dissolved were determined by HPLC (LaChrom Elite, Hitachi), utilizing a reversed-phase column C18, 250 x 4.6 mm, 5 μm (Kinetex™ - Phenomenex, France); UV-detector 252 nm; 1.0mL/min flow rate; 40 μL volume injection and mobile phase ACN: acetate buffer (60:40).

#### *Methods used to compare EFV release profiles from the cylindrical organogels*

Efavirenz (EFV) release profiles were analyzed using model-dependent (curve-fitting) and independent approaches (Dissolution Efficiency – DE). The free open source software KinetDS®<sup>33</sup> was used to fit the release curves to the Korsmeyer-Peppas, Weibull and Hixson-Crowell mathematical models [Equations (2-4), respectively, Table 1). The accuracy and prediction ability of these models were compared by calculation of coefficient of determination (R<sup>2</sup>), AIC (Akaike's information criterion) and RMSE (root mean square error).

The dissolution (or release) efficiency (DE), calculated by KinetDS® according to Equation (8) (Table 1), is a model-independent parameter that was also used to evaluate the EFV release performance under different formulations and test conditions. The differences for DE were statistically examined by one-way analysis of variance (ANOVA) followed by Tukey test, in order to find the source of difference (p<0.05). In this method, DE (*n*=2) was the dependent variable, and Formulation was the

factor. The calculations were performed using the software Statistica version 12.0 (Data Analysis Software system, StatSoft, Inc., Tulsa, OK). Throughout the study, *p* < 0.05 was used as the criterion to assess statistical significance.

#### *Organogel beads for drug delivery purpose*

**Preparation method.** Before beads production, the organogel formulation was preliminary selected. The concentration of 12-HSA was fixed at 20wt% and the drug loading at 60mg/g, an interesting composition for controlled drug release. Blank organogels (without drug) were also prepared under the same conditions for comparison. Organogel beads were generated using prilling equipment (Spherisator M, BRACE – GmbH, Germany). After melting 12-HSA in sunflower oil at 90°C, EFV was added to the melt under stirring up to its complete dissolution in the molten matrix. By applying air pressure (50 mbar), the mixture was extruded towards a thermostated nozzle (inner diameter: 0.4mm). The extruded liquid jet was broken up into droplets of equal size by the application of a vibrational frequency (250 Hz). The droplets were allowed to form and fall into a thermostated vessel containing water at 25 °C. The distance between the nozzle and the collector bath was adjusted to 90 mm. A surfactant (0.2 wt% Tween-20) was added into the collector bath to prevent the formation of bead aggregates. The beads were collected immediately, rinsed thoroughly with distilled water and then stored in dry atmosphere at ambient temperature until further experiments.

#### *Characterization methods*

**Bead size and shape.** Beads were observed by optical microscopy (OM) and scanning electron microscopy (SEM). The OM was performed by numerical microscope (Keyence VHX-5000, France) equipped with objective-glass Z20 and Z100. Concerning SEM, a XL30 ESEM-FEG equipment (Philips, USA) was utilized. At least 100 beads were analyzed from each batch. An average value for all beads has been calculated as the mean particle size.

**Raman microscopie mapping.** To further characterize the beads, complementary Raman analyzes were undertaken focusing on the evaluation of the spatial distribution of the components within the gel beads. Raman microscopie mapping was performed using the Alpha 300AR Confocal Raman-AFM microscope (Witec, Germany) equipped with a double laser Nd:Y AG at a wavelength of 532 nm. Cross-sections of beads were scanned by a 50 x long working distance objective lens, which allowed 432 nm of lateral resolution and 1320 nm of axial resolution. Ail spectra were acquired using an exposure time of 10s and a step size of 0.2 μm. The area mapped was approximately 15 μm x 30 μm. The resulting images provide information about the distribution of EFV within the organogel beads.

#### *Drug release studies from organogel beads*

Dissolution profile of the organogel beads generated by prilling, composed with 20wt% of 12-HSA and loaded with 60mg/g of Efavirenz was performed using an USP4 apparatus (Sotax CH-4008, Switzerland) equipped with three flow-through cells. Twenty beads were placed into each cell operating at a flow rate set of 16mL/min in a closed circuit. In order to simulate the GI tract, two dissolution media were successively used according to Pharmacopeia recommendations (2h at pH 1.2 and then 8h at pH 6.8). About 1wt% SDS was added in the media as previously described. According to the literature, the equilibrium concentration of the drug in simulated gastrointestinal fluid containing 1wt% is close to 2.5 mg/g<sub>solution</sub> at 37 °C<sup>34</sup>. The drug



Table 1. Dependent and independent kinetic models applied to analyze the drug release

Approach	Method	Equations
Model-dependent	Korsmeyer-Peppas	$Mt = KKP.t^n$ (2)
	Weibull	$Mt = 1 - \exp(- (Ti)^b)$ (3)
	Hixson-Crowell	$(1 - Mt/Mo)^{1/3} = KHc.t$ (4)
	Higuchi	$Mt = KHi.t^{0.5}$ NE(5)
	Best mode/ criteria	$RMSE = \sqrt{\frac{1}{n} \sum (y_{iobs} - y_{ipred})^2}$ (6)
		$AIC = 2k + n \cdot \ln \left( \frac{1}{n} \sum (y_{iobs} - y_{ipred})^2 \right)$ (7)
Model-Independent	Dissolution efficiency (DE)	$DE = \left( \frac{\sum Mt}{M_{max}.t} \right) \times 100$ (8)

$Mt$ : amount of drug released in time  $t$ .

$Mo$ : initial amount of drug in the dosage form.

$KHi$  is the release constant in Higuchi mode!.

$KHc$  is the release constant in Hixson-Crowell mode!.

$KKP$  is the release constant incorporating structural and geometric characteristics of the drug-dosage for;  $n$  is the diffusional exponent indicating the drug-release mechanism.

$M_{max}$ =maximum amount of drug released (=100%).

$N$ =number of time points.

$Ti$ =lag time before the onset of the release process (in most cases will be zero).

$a$ =time scale of the process.

$b$ =shape parameter.

concentration used in the dissolution tests was 0.04 mg/g solution. Samples were withdrawn at predefined time intervals up to 10h and directly analyzed. The amount of drug released at each time point was determined by the previously described HPLC-UV method. All experiments were performed in triplicate.

The free open source software KinetDS®<sup>25</sup> was used to fit the release curves to the Korsmeyer-Peppas, Weibull, Hixson-Crowell and Higuchi mathematical models [Equations (2-5), Table 1]. The accuracy and prediction ability of these models were compared by calculation of coefficient of determination ( $R^2$ ), AIC and RMSE.

## Results and discussion

### Drug solubility in molten organogels

Organogels were prepared for prilling at 90 °C. The determination of the drug solubility in the molten organogel (for the highest organogelator concentration tested) was necessary to ensure the complete dissolution of the drug in the molten organogel and prevent nozzle clogging during prilling.

Solution calorimetry measured the heat change generated from the dissolution of the solid EFV by the molten organogel constituted of sunflower oil and 20 wt% of 12-HSA. The creation of a saturated solution at 90 °C involved dissolving the solute (EFV) up to the maximum and constant concentration that also corresponds to a constant value of heat of solution. As shown in Figure 1, incremental amounts of EFV added to the molten organogel resulted in a straight line increase in the magnitude of the heat of solution until a plateau was reached when the concentration of the solute equaled the saturation point. From Figure 1, the EFV mass where the two straight lines cross corresponds to the amount of solute that saturates the solvent contents in the vessel. The solubility value corresponds to 75 mg/g for EFV in the molten organogel at 90 °C. From these results, we choose to work with a concentration below the saturation (30 and 60 mg of EFV per gram of organogel).

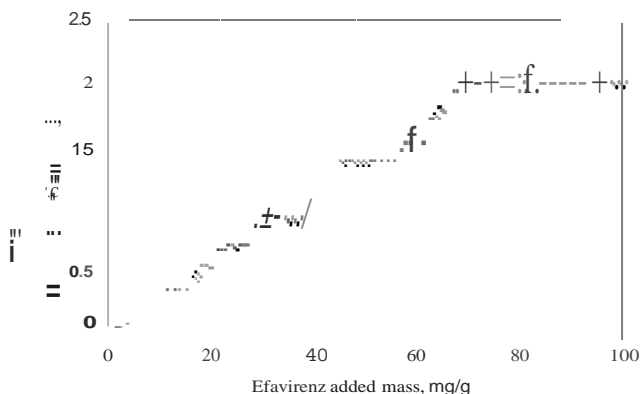


Figure 1. Enthalpy of dissolution of EFV in molten organogel comprising 12-HSA (20 wt%) and sunflower oil at 90 °C. Means with error bars for two experiments ( $n=2$ ) are shown.

It is also important at this stage to keep in mind the organogel concept, in which oil is entrapped in a network self-assembly of the gelator molecules creating a gel. In this study, sunflower oil is entrapped in the self-assembled fibrillar networks of 12-HSA. The aim is to entrap EFV amounts smaller than its solubility limits at 37 °C in the oil phase in order to keep the drug always dissolved when exposed to the release medium. This condition is achieved for the EFV concentrations equal or lower than 60 mg per gram of organogel containing 20% 12-HSA (corresponding to 75 mg per gram of sunflower oil), since the equilibrium concentration determined experimentally after 72 h is equal to  $79.8 \pm 0.6$  mg of EFV per gram of sunflower oil as shown in Table 2.

### Thermal analysis

The effects of the gelator concentration and the drug incorporation on the thermal properties of the organogels were studied by DSC. DSC thermograms for the second

Table 2. EFV equilibrium concentration in sunflower oil at  $37 \pm 1^\circ\text{C}$  as a fonction of time.

Equilibrium lime (h)	24	48	72	Median value*
EFV concentration (mg/g oil)	$82.47 \pm 2.18$	$79.14 \pm 3.49$	$79.78 \pm 0.65$	80.02

\*The median of ail measured values from 24 to 72h because we cannot see differences among concentrations al different limes, the differences being smaller than standard deviations.

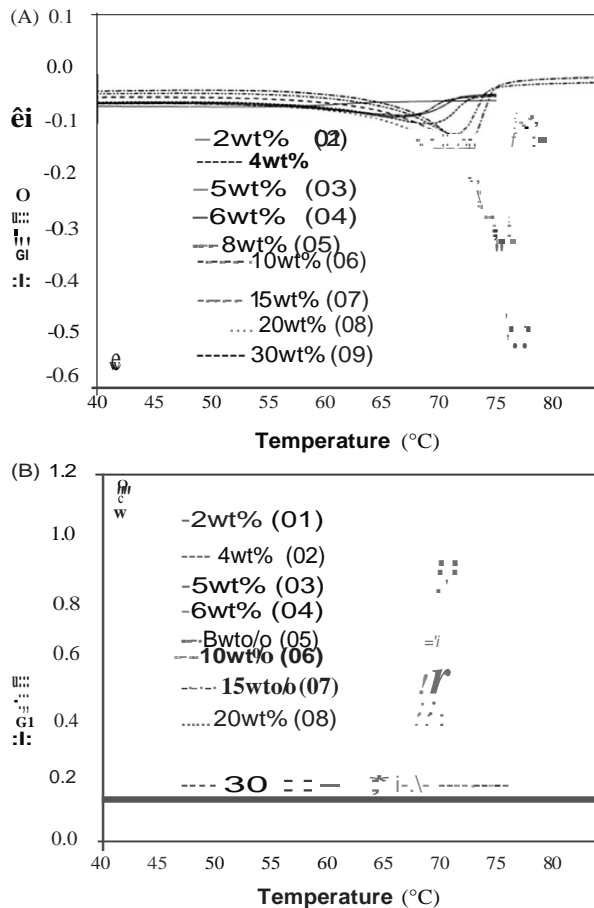


Figure 2. DSC second-heating for gelled sunflower oil over the range of 12-HSA compositions from 2% to 30wt%. Melting (A) and Crystallization (B).

heating-cooling cycle of the organogels without EFV (Samples 01-09) are shown in Figure 2(a) and (b), respectively. The position of the melting and the crystallization peaks shifts to higher temperatures as the concentration of 12-HSA is increased and they become broader and shallower at low concentrations of 12-HSA as shown in the bottom curves in Figure 2(a) (melting) and in the top curves in Figure 2(b) (crystallization). As expected, the organogels were thermo-reversible as identical thermal parameters were obtained over the two melting/crystallization cycles (data not shown).

Table 3 shows the thermal parameters that describe the crystallization and melting behavior of organogels over the range of compositions from 2% to 30% (w/w) HSA.  $T_{m(\text{onset})}$ ,  $T_m$  and  $T_{m(\text{endset})}$  are the temperatures at the beginning, peak and end of the melting endotherm, respectively.  $T_{c(\text{onset})}$ ,  $T_c$  and  $T_{c(\text{endset})}$  are the temperatures at the beginning, peak and end of the crystallization exotherm, respectively.  $AH_m$  and  $AH_c$  are the melting and crystallization enthalpies, respectively, determined by integrating the area of each peak, melting and crystallization respectively.

The thermal point of technical-grade 12-HSA is roughly  $76^\circ\text{C}$  with a melting enthalpy of  $182\text{J/g}$ <sup>35</sup>. 12-HSA/sunflower organogels display the following melting characteristics: as gelator concentration is increased, so does the melting enthalpy and the onset of melting, i.e. the gel melts at a higher temperature. For example, a 2wt% HSA sunflower oil gel has a  $T_m$  of approximately  $57^\circ\text{C}$ , against  $75^\circ\text{C}$  for a 20 wt% HSA. It is perhaps appropriate to remember that the main goal of this study is the optimization of a structured organogel matrix in which EFV could be solubilized to control its delivery in the GI tract after oral administration. Thus, the best formulation for EFV-loaded organogels should take into account the melting temperature of these systems. In fact, organogels being thermoreversible, they should guarantee both the thermal stability and solid-like behavior at a temperature higher than or equal to the human body temperature, and even higher than  $40^\circ\text{C}$  to combine environmental and storage aspects. All fractions of 12-HSA used in this study to produce sunflower oil gels are able to lead to a structured network of 12-HSA since the onset for melting is approximately  $48^\circ\text{C}$  for the lower gelator concentration (2wt%).

Besides the gelator and its concentration, the effect of the drug on the gel structures should be an important parameter to be taken into account when developing organogels for drug delivery purposes. In previous studies reported in the literature, the drug was mostly suspended in the gel medium and had a little or no impact on the structures of the drug-loaded gels, which possessed nearly identical physical properties with blank gels<sup>15,17</sup>. In other cases, although no impact was observed on the gelation temperature, the presence of some dissolved drug in the gel apparently shortened the time of phase inversion while raising drug content<sup>36</sup>.

The effect of the drug incorporation in the thermal transition temperatures of the organogels was also studied by DSC. EFV was incorporated (30 mg/g<sub>organogel</sub> or 60 mg/g<sub>organogel</sub>) in 12-HSA/sunflower oil gels containing different gelator concentrations (5%, 10%, 20% and 30wt%). The DSC thermograms for the second heating-cooling cycle of EFV-loaded organogels formed with 20% wt HSA are shown in Figure 3(a) and (b). The same trend was systematically seen for the whole gelator concentrations studied. The temperatures and heats of melting (i.e.  $T_m$  and  $AH_m$ ) and crystallization ( $T_c$  and  $AH_c$ ) are reported in Table 3.

The organogel melting enthalpy ( $AH_m$ ) combines at least two distinct energetic contributions: (1) the melting enthalpy of the fibers (which should be more or less constant and independent of the solvent used if the fibers comprise pure gelator molecules organized in a similar and regular structure) and (2) the enthalpy of dissolution of the gelator in the solvent phase, which is related to the molecular affinity between the two species and should depend on the solvent used<sup>37/38</sup>. A decrease of the melting enthalpy could be taken as a sign of some re-arrangements in the microstructure and/or packing of the 12-HSA molecules occurring in the presence of the dissolved drug in the oil. However, the data shown in Table 3 suggest that the introduction of EFV in the gelling system did not result in evident changes on  $AH_m$  or  $AH_c$  of the formulations, but apparently reduced the thermal transition temperatures ( $T_m$ ,  $T_c$ ) by a few degrees while raising drug content. This is quite visualized for low gelator concentrations, the differences decreased when the HSA fraction increased, both  $T_m$

Table 3. Thermal parameters obtained from the melting and crystallization thermograms of organogel with different concentrations of 12-HSA in sunflower oil, neat and loaded with EFV.

Organogel composition and conditions of preparation				Thermal properties measured by DSC							
Sample ID	HSA fraction (w/w)	Sunflower oil (w/w)	EFV (mg/gorganoe1)	Melting ( $T_m$ )*				Crystallization ( $T_c$ )*			
				Onset (°C)	Peak (°C)	Endset (°C)	$\delta H_m$ (J/g)	Onset (°C)	Peak (°C)	Endset (°C)	$\delta H_c$ (J/g)
O1	0.02	0.98		48.3 (0.9)	56.6 (0.7)	61.4 (0.8)	0.75 (0.1)	54.0 (0.4)	51.0 (0.4)	43.6 (0.6)	-0.54 (0.1)
O2	0.04	0.96		53.9 (0.1)	61.5 (0.1)	67.7 (0.4)	0.90 (0.1)	57.7 (0.2)	55.0 (0.5)	49.6 (0.6)	-0.80 (0.1)
O3	0.05	0.95		55.5 (0.4)	66.9 (0.1)	70.7 (0.1)	4.5 (0.1)	63.7 (0.1)	62.2 (0.1)	59.2 (0.2)	-5.2 (0.5)
O4	0.06	0.94		55.01 (0.4)	67.3 (0.3)	72.2 (0.4)	7.5 (0.1)	65.4 (0.2)	63.6 (0.1)	57.5 (1.5)	-8.1 (0.3)
O5	0.08	0.92		59.9 (0.9)	69.1 (0.4)	72.8 (0.2)	8.4 (1.1)	66.0 (0.1)	64.5 (0.1)	61.3 (0.5)	-9.3 (0.1)
O6	0.10	0.90		64.5 (0.1)	71.8 (0.5)	74.4 (0.4)	12.1 (0.9)	68.6 (0.1)	65.4 (0.5)	62.7 (2.2)	-13.5 (0.1)
O7	0.15	0.85		66.2 (0.8)	72.2 (0.2)	75.2 (0.7)	16.3 (0.7)	68.5 (0.2)	67.6 (0.1)	66.1 (0.1)	-15.9 (0.1)
O8	0.20	0.80		72.3 (2.5)	75.3 (0.2)	77.05 (0.8)	29.6 (1.7)	70.2 (0.1)	68.8 (0.1)	67.5 (0.1)	-26.3 (0.1)
O9	0.30	0.70		74.2 (2.1)	77.4 (1.1)	78.6 (0.8)	41.2 (2.1)	71.0 (0.16)	70.5 (0.1)	68.7 (0.1)	-41.2 (0.3)
OEFV1	0.05	0.95	30	55.2 (6.5)	62.5 (0.4)	70.8 (3.0)	5.1 (0.1)	61.0 (0.4)	58.8 (0.1)	55.5 (1.2)	-4.8 (0.2)
OEFV2	0.10	0.90	30	60.2 (0.4)	69.7 (0.6)	73.2 (0.3)	11.0 (0.2)	66.6 (0.1)	64.2 (0.4)	60.2 (0.7)	-10.1 (0.9)
OEFV3	0.20	0.80	30	68.4 (1.0)	74.3 (1.1)	76.2 (0.6)	28.5 (1.1)	68.9 (0.3)	67.2 (0.1)	64.9 (0.2)	-27.7 (0.1)
OEFV4	0.30	0.70	30	72.8 (2.5)	75.6 (0.3)	77.1 (0.2)	41.4 (1.5)	70.3 (0.1)	69.6 (0.1)	68.0 (0.1)	-39.0 (0.5)
OEFV5	0.05	0.95	60	50.9 (5.9)	58.0 (2.6)	61.8 (5.1)	5.7 (0.4)	56.0 (0.8)	52.0 (1.5)	47.4 (0.2)	-5.0 (0.3)
OEFY6	0.10	0.90	60	59.2 (0.2)	69.4 (1.1)	72.0 (0.3)	13.7 (1.0)	62.6 (0.1)	61.1 (0.6)	59.1 (0.6)	-13.9 (0.1)
OEFY7	0.20	0.80	60	70.0 (4.4)	73.6 (1.0)	75.01 (0.3)	27.6 (1.2)	67.1 (0.1)	65.2 (0.1)	64.6 (0.6)	-28.3 (0.1)
OEFV8	0.30	0.70	60	72.0 (2.8)	74.8 (0.1)	76.6 (0.1)	40.4 (1.4)	69.7 (0.4)	68.2 (0.1)	66.7 (0.2)	-38.8 (0.3)

\*Standard errors in brackets.

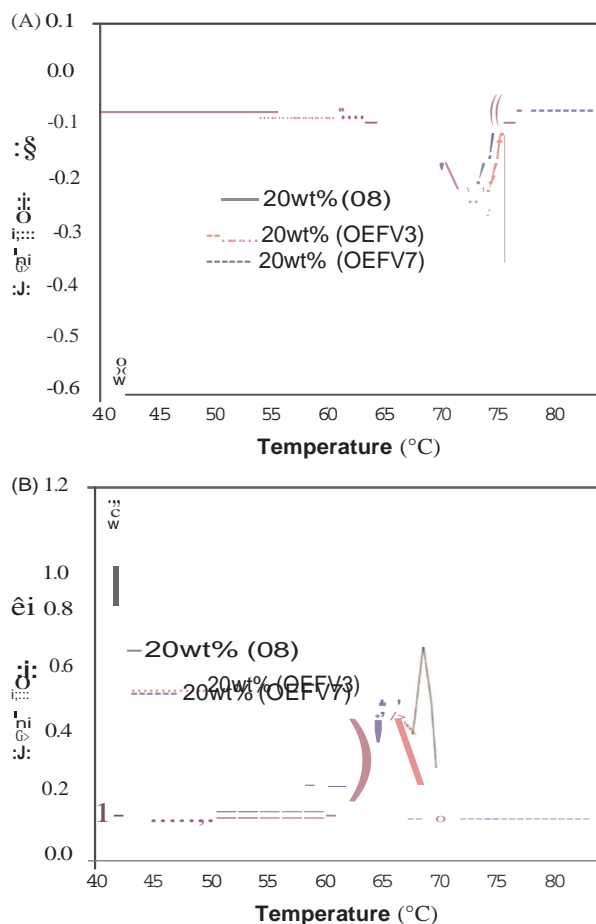


Figure 3. DSC second-heating for gelled 12-HSA (20wt%) – sunflower oil loaded with EFV (30mg/gorganogel or 60mg/gorganogel). Melting (A) and Crystallization (B).

and  $T_c$  are almost constant and only function of the gelator amount. A similar effect in onset temperatures was reported for soy organogel with different gelators: myristic, palmitic, stearic and arachidic acids<sup>39</sup>.

The onset of  $T_c$  is the last point associated with the solution state and the onset of  $T_m$  is the last point of the gel state. Anyway, the peak melting  $T_m$  and onset of crystallization temperatures of the gels increased as 12-HSA concentrations increased, whatever the gel studied, in the presence and absence of drug. Both thermal parameters scaled in a logarithmic fashion in relation to the 12-HSA concentration (Figure 4). The sol-gel transition is sometimes interpreted as dissolution or melting of the "crystal" gelling molecules. In this sense, the solubility of "crystals gelling" corresponds to the concentration of gelling agent in an ideal solution at a given temperature by the following Equation (9), called Schroeder-van Laar equation<sup>37,40,41</sup>.

$$L \ln C = \frac{-\delta H_m}{R \cdot T_m} + \text{constant } e \quad (9)$$

Where  $R$  is the universal gas constant and  $\delta H_m$  corresponds to the melting enthalpy apparent (sol-gel) of the pure gelling and  $C$  its molar concentration in the gel. According to this equation, a plot of  $L \ln C$  versus  $1/T_m$  should be a straight line. This linear dependency is confirmed in Figure 5. The melting and crystallization enthalpies likewise increased in a linear manner with an increase of the 12-HSA concentration (Figure 6). Similar behaviors were already reported in the literature using the 12-HSA dissolved in various organic phases<sup>42</sup>.

#### Organogel erosion and drug release kinetics (cylindrical shape)

To clarify the mechanism of drug release from organogels, the weight loss and the amount of drug released were measured as a function of time. Experimental data plotted in Figure 7 highlights an initial slow release during the first hour, during which probably the drug diffuses slowly, followed by a second step characterized by a faster release. This faster release could be attributed to the onset of an erosion phenomenon, which makes the entrapped drug

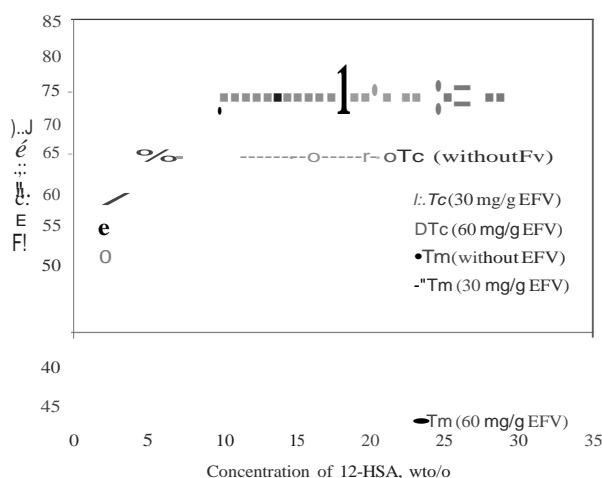


Figure 4. Transition phase diagrams of organogels in different 12-HSA concentrations made without active pharmaceutical ingredient, with 30mg/g and 60mg/g of Efavirenz. Blank symbols correspond to crystallization and black symbols correspond to melting. Means with error bars for two experiments ( $n=2$ ) are shown.

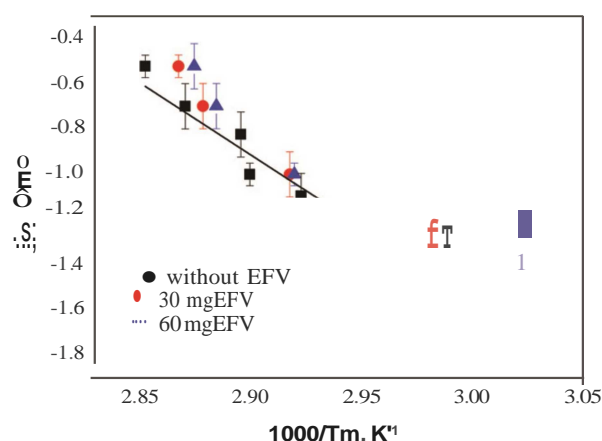


Figure 5. Representation of Schroeder-van Laar equation for the analysis of the influence of gelling agent concentration on the sol-gel transition temperature. Means with error bars for two experiments ( $n=2$ ) are shown.

more available. Combined drug diffusion and erosion may contribute to a faster release than erosion alone<sup>16</sup>.

The data shown in Figure 7 include the influence of four different parameters: gelator concentration (5 or 20 wt% HSA), temperature of gel formation (5 °C or 25 °C), and composition of the release media (pH 1.2 containing pepsin or pH 6.8 containing pancreatin) to simulate the GI tract.

At the end of 8h of release, the mass lost (erosion) and the amount of drug released were more pronounced for organogels less concentrated in 12-HSA (5 wt%) formed at lower temperature (5 °C). In fact, organogels containing 5 wt% HSA (OEFV1) formed at 5 °C released 34% of EFV under simulated gastric conditions against 51% in simulated intestinal fluid. The corresponding organogel erosion percents were 25 and 41%, respectively. The temperature of gel formation influenced the amount of drug released and the organogel mass loss. Increasing the temperature of organogel formation to 25 °C led to an increased drug release (36% of EFV released in gastric and 45% in intestinal conditions; 27 and 39% of organogel mass loss, respectively).

In turn, organogels formed at 25 °C, loaded with 20 wt% 12-HSA (OEFV7) provided the smaller amounts of drug released and mass losses. They released approximately 24% of EFV under simulated gastric conditions against 32% in simulated intestinal

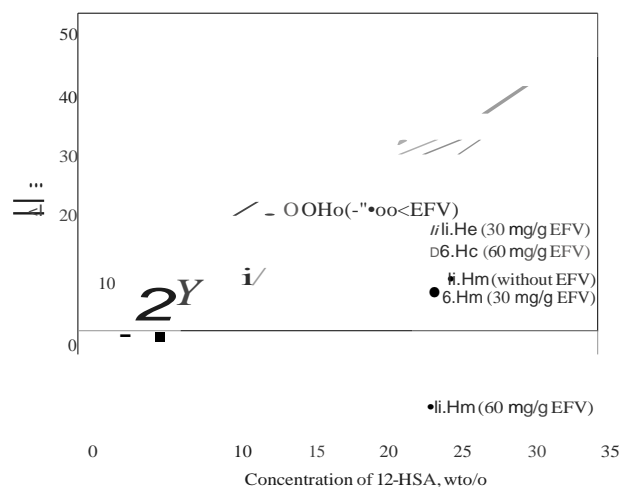


Figure 6. Enthalpy of melting and crystallization of 12-HSA calculated from DSC peak area for 12-HSA series (organogels without active pharmaceutical ingredient and with 30 or 60mg/g of Efavirenz). Blank symbols correspond to crystallization and black symbols correspond to melting. Means with error bars for two experiments ( $n=2$ ) are shown.

fluid. The corresponding organogel erosion percents were 4 and 12%, respectively. It was also observed that the same organogels when formed at 5 °C eroded more easily (7 and 22% mass losses in gastric and intestinal conditions, respectively), although minor differences were observed on the amounts of drug released (24 and 34%), compared to those at 25 °C.

In summary, the data presented here evidenced that both erosion and drug diffusion from the 12-HSA sunflower organogel are dependent on the matrix network (directly correlated to HSA concentration and temperature of gel formation) and slightly affected by the EFV presence, especially at low concentration of HSA. Therefore, the more structured the system the lower should be the matrix erosion, yielding to a lower drug release as a final result. More structured gels were obtained with the higher gelator concentration (20 wt% HSA) and the higher temperature of gel formation (25 °C). Both erosion and drug diffusion from more structured gels were less affected by the pH of the medium. These findings are also in agreement with the few works found in the literature related to drug delivery from organogels in simulated gastric and intestinal test solutions<sup>15,17</sup>.

## Release profiles from the cylindrical organogels

### Dependent models (curve fitting)

In a general manner, the drug release from organogels may involve processes of diffusion, erosion or drug dissolution<sup>16,17</sup>. Diffusion and erosion are expected to be the processes controlling drug release from the organogels since EFV at the concentrations tested, is completely dissolved within the organogels, as expected from the measured solubility data and confirmed by DSC studies. The drug is then immediately available for diffusion.

A large number of models (both empirical and mechanistic mathematical models) have been proposed to describe drug release mainly for polymer matrices characterized by a 3D network. Even though organogels have different structures, the models already proposed for more investigated polymeric carriers can be useful to fit experimental values to compare the release profiles obtained from different formulation (gelator concentration, EFV loading) and conditions (gel formation temperature) studied here.

The *in vitro* drug release data from the different organogels containing EFV were evaluated kinetically using the Korsmeyer-Peppas, Weibull and Hixson-Crowell models [Equations (2-4), Table 1]. Korsmeyer-Peppas model is expected to be valid only up to 60% cumulative drug release, so the data for analysis were restricted to this range. The coefficient of



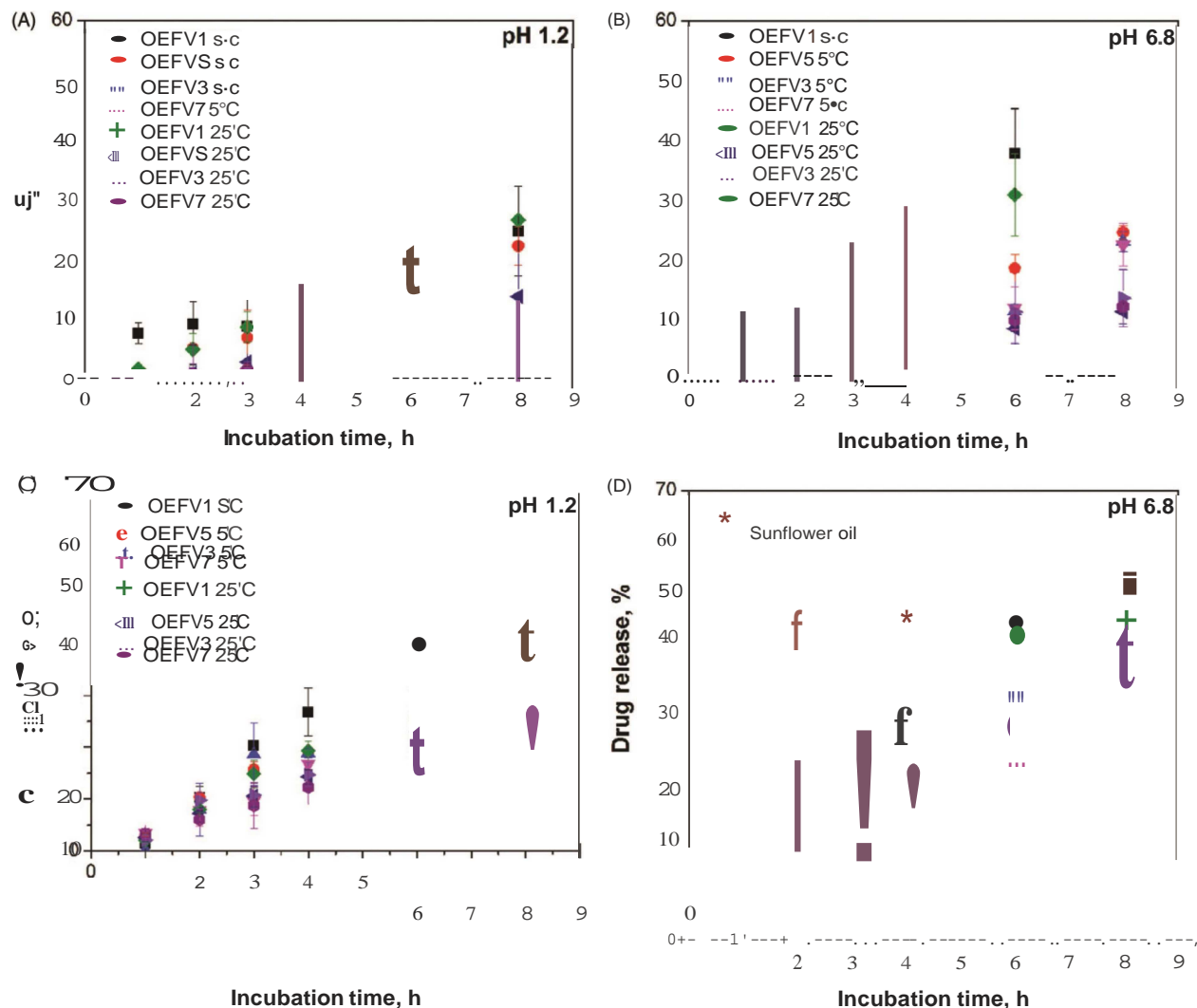


Figure 7. Weight loss and release studies of organogels containing EFV at 37 °C. (a, c) Simulated gastric solution at pH 1.2 including pepsin 800 U/L enzyme activity; (b, d) Simulated intestinal solution at pH 6.8 including pancreatin 1750 U/L protease activity. Means with error bars for two experiments ( $n=2$ ) are shown.

determination ( $R^2$ ), root-mean-square error (RMSE) and Akaike's information criterion (AIC) values of these models were determined for evaluation of accuracy and prediction ability of these models using KinetDS 3.0 rev. 2010 software (Krakow, Poland). The result of the curve fitting into various mathematical models is given in Table 4. The best fitting of models was based on goodness of fit, i.e. the highest coefficient of determination ( $R^2$ ) (closer to unity) and lowest RMSE and AIC values (definition in Table 1).

From the obtained results, it is observed that for all formulations the drug release mechanism is best described by Korsmeyer-Peppas model. This model is a semi-empirical model, relating exponentially the drug release to the elapsed time. It is used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved<sup>43</sup>.

The release exponent ( $n$ ) is used in order to characterize different release mechanism. If the  $n$  value is 0.5 or less, the release mechanism follows Fickian diffusion. Higher values of  $n$  ( $0.5 < n < 1$ ) correspond to a non-Fickian model denominated anomalous transport. The drug release follows Zero-order drug release or Case-II transport if the  $n$  value is 1. For the values of  $n$  higher than 1, the mechanism of drug release is regarded as super Case-II transport, describing the influence of polymeric hydration and swelling on drug release for polymeric and swellable systems, and which can be related to matrix erosion for non-swellable systems.

The values of diffusional exponent ( $n$ ) determined from Korsmeyer-Peppas model ranged from 0.81 to 1.39 (Table 4) for all organogels loaded with EFV (FI-FI6, refer Table 5 for formulation code description), indicating that the drug release from these systems followed the super case-II transport mechanism. However, the  $n$  value determined from this model for Reference (when EFV-60 mg/g<sub>oil</sub> is dissolved in non-gelled oil) is 0.30 (FI7, Table 4), denoting a Fickian diffusion mechanism. In conclusion, the release mechanism from organogels loaded with EFV is different from the diffusion mechanism obtained with simple oil formulations. From organogels, EFV probably diffuses through an outer gel layer that erodes, releasing oil droplets containing dissolved EFV into the aqueous medium. This conclusion is supported by the visual observation of the cylindrical organogels before and after release in the test solutions. The photographs shown in Figure 8 were taken at the end of the erosion/release studies. It is clearly visible that the organogels undergo changes in thickness (decrease in diameter) mainly for organogels containing 5 wt% HSA, while the capsule length remained essentially unchanged. These findings evidence erosion occurring at the organogel surface (Figure 8) depending on the gelator concentration.

The results from the evaluation of drug release mechanism using various kinetic models confirmed the experimental observations that both erosion and drug diffusion mechanisms take place, depending on the matrix network.

Table 4. Results of curve fitting of the *in vitro* release data from different cylindrical organogels containing EFV.

		Formulation code (Table 5)								
Model-dependent method	Kinetic parameters	FI	F2	F3	F4	F9	F10	Fi 1	FI2	
Test solution: gastric solution (H 1.2) at 37 °C comprising pepsin										
Korshmeier-Peppas	R	0.9992	0.9999	0.9993	0.9999	0.9993	0.9997	0.9534	0.9998	
	RMSE	1.2990	0.8705	1.8703	0.2703	1.2988	1.2349	2.6259	1.3810	
	AIC	21.28	15.68	26.38	-0.69	21.28	20.57	31.14	22.14	
	K	4.33	3.67	4.25	2.90	4.33	5.84	4.82	4.64	
	n	1.02	0.98	0.93	1.05	1.02	0.82	0.96	0.81	
Weibull	DR (%)4h	17.81	14.28	15.51	12.45	17.81	18.18	18.28	14.21	
	R2	0.9990	0.9998	0.9992	0.9999	0.9990	0.9997	0.9520	0.9998	
	RMSE	2.5639	1.0580	2.0647	0.6355	2.5639	1.5196	11.019	1.3133	
	AIC	30.80	18.41	27.71	11.27	30.69	23.48	51.22	21.43	
	R2	0.8024	0.7844	0.7692	0.7854	0.8024	0.7306	0.7625	0.7186	
Hixson-Crowell	RMSE	7.5353	5.8859	6.8725	4.5060	7.5354	7.8429	10.248	6.101	
	AIC	45.89	42.44	4.46	38.70	45.89	46.46	50.20	42.94	
Model-independent method	DE	17.68	14.36	15.57	12.65	17.68	17.54	17.83	13.92	
	Number of time points	7	7	7	7	7	7	7	7	
Test solution: intestinal solution (pH 6.8) at 37 °C comprising pancreatin										
		Formulation code (Table 5)								
Korshmeier-Peppas	R2	0.9989	0.9999	0.9993	0.9992	0.9997	0.9998	0.9998	0.9997	0.9975
	RMSE	1.0503	1.2482	1.5821	0.7897	1.2178	1.5781	1.6493	1.5383	3.4737
	AIC	18.3	2.07	24.04	14.32	20.38	24.08	24.63	23.65	24.45
	K	5.48	4.52	7.82	3.37	9.01	4.10	4.21	1.83	29.20
	n	1.02	0.89	0.65	1.07	0.85	0.99	1.05	1.39	0.30
	DR (%)4h	22.55	15.46	19.22	14.94	29.24	16.16	18.03	12.63	44.00
Weibull	R2	0.9999	0.9998	0.9994	0.9987	0.9999	0.9998	0.9997	0.9995	0.9980
	RMSE	1.1189	1.2339	1.5218	1.3811	0.9910	1.6369	2.057	3.4710	3.251
	AIC	19.19	20.56	2.35	22.14	17.49	24.52	27.72	35.04	23.84
	Rz	0.7726	0.7573	0.6457	0.7931	0.7228	0.7665	0.7890	0.8352	0.4353
Hixson-Crowell	RMSE	8.199	6.6980	8.0967	8.4566	11.380	14.1924	18.713	18.8616	21.05
	AIC	47.08	44.25	46.90	41.38	53.67	43.15	44.81	36.54	42.51
Model-independent method	DE	22.86	15.33	18.04	15.20	28.64	16.18	18.44	13.95	40.74
	Number of time points	7	7	7	7	7	7	7	7	5

Table 5. Comparison of EFV-loaded organogel release profiles using the independent mode! values of dissolution efficiency DE (done using one-way [ANOVA] followed by Tukey's test).

Formulation code	Formation temperature	EFV mg	HSA%	pH	Dissolution efficiency, DE mean (n=2)*
F17 (oil formulation)	25	60	0	6.8	40.38"
F14	5	60	5	6.8	30.97•b
F13	5	30	5	6.8	29.07•b
FS	25	30	5	6.8	22.86a,b
F9	5	30	5	1.2	21.46•b
FIS	5	30	20	6.8	19.29•b
F7	25	30	20	6.8	18.04b
FI	25	30	5	1.2	17.68b
F11	5	30	20	1.2	17.43b
F10	5	60	5	1.2	17.43b
F3	25	30	20	1.2	15.57b
F8	25	60	20	6.8	15.53b
F6	25	60	5	6.8	15.33b
F16	5	60	20	6.8	14.46b
F2	25	60	5	1.2	14.36b
F12	5	60	20	1.2	14.17b
F4	25	60	20	1.2	12.65b

\*Values represent the mean of two independent determinations. Same letters at the same column indicate that there is no statistical difference ( $p < 0.05$ ).

#### Independent model

Dissolution efficiency (DE) results are shown in Table 5. The analysis of variance (ANOVA) revealed a statistical difference ( $p < 0.05$ ) between the formulations. Differences in the DE

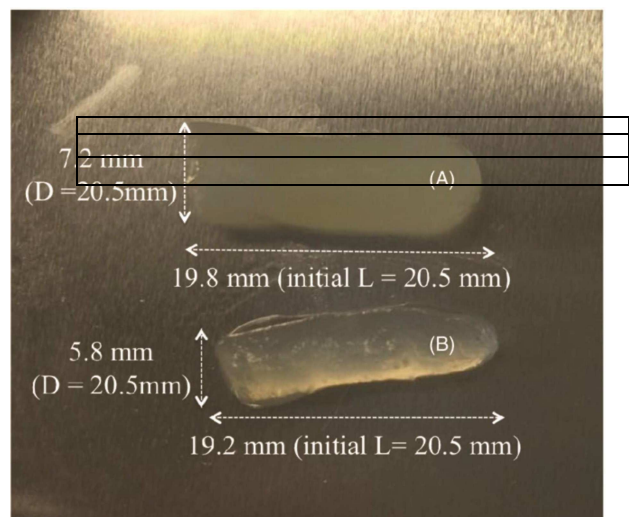


Figure 8. 12-HSA sunflower oil gel formed in gelatin capsules (size #00) removed after 8 h of incubation into simulated intestinal solution at 37 °C, pH 6.8 including pancreatin 1750 U/L protease activity. (A) 20 wt% HSA and (B) 5 wt% HSA. Both organogels were loaded with EFV (60 mg/g). A calibrated vernier caliper was used for diameter and thickness evaluation of the gel capsules.

data may be related to the release profiles since factors such as 12-HSA concentration, drug loading, gel formation temperature and pH of the release medium may influence the release of the drug molecule from the organogels.

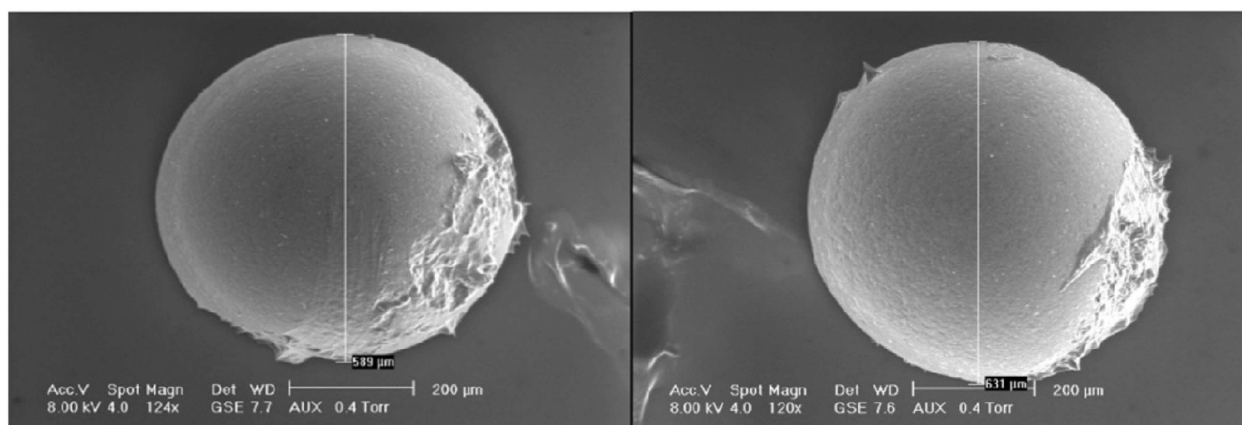


Figure 9. SEM image of organogel beads produced by prilling process.

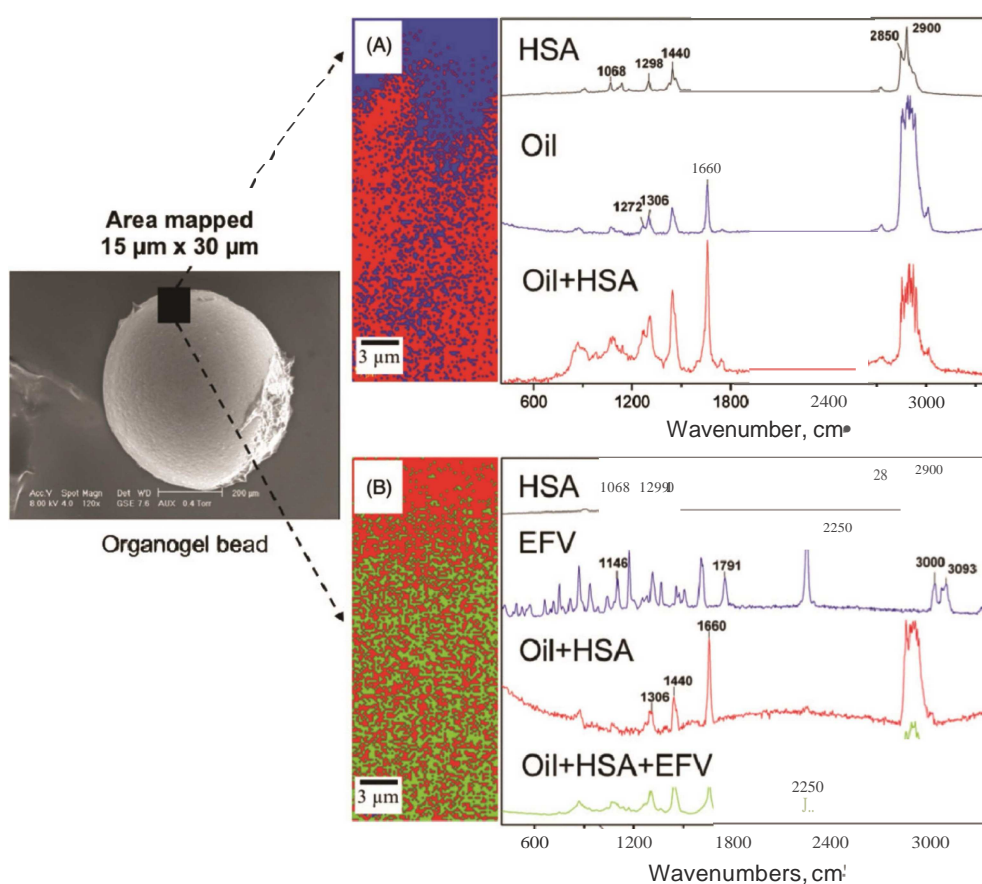


Figure 10. Raman spectra and mapping for EFV-loaded organogel beads.

To evaluate the difference among the 17 formulations, a *post hoc* Tukey test was performed on the results of ANOVA. With the application of the Tukey test it was possible to observe that, with a level of significance of 5%, DE of formulations F1, F2, F3, F4, F6, F7, F8, F10, F11, F12 and F16 were equal and significantly smaller than the DE found for the formulation F17. Moreover, F17 is not significantly different from F5, F9, F13, F14 and F15 ( $p>0.05$ ), which are not significantly different from one other.

From these results, it could be concluded that the drug release was delayed by the gelled oil as already discussed, i.e. an oil-structured system is expected to present lower drug release rate.

However, the different parameters studied such as the gelator concentration, the drug loading, the temperature of organogel formation and even the pH of the release media showed no significant difference on DE.

## Organogel beads for drug delivery purposes

### Beads characterization

**Size and shape.** Figure 9 reveals that the organogel beads consist of large micron-scale beads, spherical in shape and very uniform in diameter ( $610 \pm 5 \mu\text{m}$ ).

Table 6. Results of curve fitting of the *in vitro* release data from organogel beads containing EFV.

Test conditions: media at 37 °C containing 1wt% SDS with changing pH values (2h at pH 1.2 and then 8h at pH 6.8), without enzyme				
Best model/criteria	Model-dependent method			
	Higuchi	Hixson-Crowell	Korsmeyer-Peppas	Weibull
R2	0.9750	0.8672	0.9983	0.9985
RMSE	3.1759	7.9909	1.5272	1.9161
AJC	61.55	83.70	31.39	54.52
Parameters of the Korsmeyer-Peppas model[Equation (2)]			$k(h^n)$ =26.72 ± 4.34 $n$ =0.62 ± 0.03	
Dissolution efficiency (DE, %)	Model-independent method			
	56.38 (number of time points = 13)			

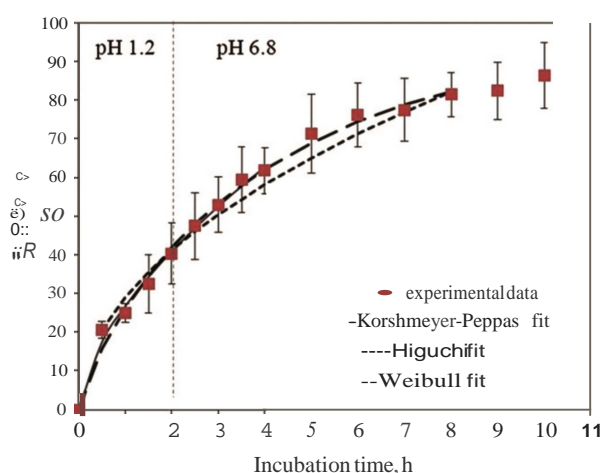


Figure 11. Release amount of EFV from the organogel beads containing 20% wt 12-HSA as gelling agent during the release test at 37 °C (2h in pH 1.2, 8h in pH 6.8). Means with error bars for three experiments ( $n=3$ ) are shown.

**Raman mapping.** The Raman spectra of the individual components (HSA, sunflower oil and organogel beads) are compared in Figure 10. The Raman spectrum of 12-HSA displays a strong peak at around 2850-2900 $\text{cm}^{-1}$  (C-H), peaks at 1440 $\text{cm}^{-1}$  ( $\text{CH}_2$ ) and two peaks around 1068-1135  $\text{cm}^{-1}$  characteristics of C-O stretching bands. In turn, the characteristic peaks are identified in the Raman spectrum of the sunflower oil: at 1660 $\text{cm}^{-1}$  (C=C stretching bands) and the peaks at 1306 and 1272 $\text{cm}^{-1}$  (=C-H deformation), already reported in the literature<sup>44</sup>. For the Raman spectrum of pure EFV, the CF<sub>3</sub> stretching modes have been assigned at 1146 $\text{cm}^{-1}$ , with a typical C=O stretching bands at 1791 $\text{cm}^{-1}$ , one distinguishing intensive band at 2250 $\text{cm}^{-1}$  assigned to C-C stretching bands, which was not evident in the other gel components.

The pure component Raman spectrum of 12-HSA could not be separated from that of sunflower oil using cluster analysis due to the homogeneous (molecular) dispersion of EFV within the organogel. Comparison of the Raman spectra of the samples revealed that, except for the EFV vibrational bands at 2250 $\text{cm}^{-1}$ , there were no distinguishable vibrations bands. EFV was found uniformly distributed within the organogel.

#### Release studies from organogel beads

Figure 11 shows the cumulative amount of EFV release profiles from organogel beads comprising 20wt% of 12-HSA and loaded with 60mg/g of EFV. The organogel beads exhibited a slow dissolution profile with 80% of the drug being released after 8h,

40% during the first 2h under simulated gastric conditions (pH 1.2). Despite an increase in the EFV solubility at pH 6.8<sup>45</sup>, drug dissolution exhibited a slow release reaching 80% after 4h of immersion into the simulated intestinal fluid.

The data obtained during the release test were examined according to Korsmeyer-Peppas, Weibull, Hixson-Crowell and Higuchi equations [Equations (2-5), respectively, Table 1]. The coefficient of determination ( $R^2$ ), root-mean-square error (RMSE) and Akaike's information criterion (AIC) values of these models were determined for evaluation of accuracy and prediction ability of these models using KinetDS 3.0 rev. 2010 software. The result of the curve fitting into various mathematical models is given in Table 6. Figure 10 also confirms the correlation of the EFV release profile with the theoretical profile predicted by these models (see fitting curves). The best fitting was based on goodness of fit, i.e. the highest coefficient of determination ( $R^2$ ; closer to unity) and lowest RMSE and AIC values (Table 1). From the obtained results, it is observed that the drug release mechanism is also best described by Korsmeyer-Peppas model. The release exponent  $n$  calculated from Korsmeyer-Peppas model for predicting drug release mechanism was 0.62, corresponding to a non-Fickian model denominated anomalous transport, revealing that the EFV release is strongly influenced by the size of the organogel system. For the organogel beads an anomalous transport is the main release mechanism, whereas for cylindrical organogels (larger size) erosion and diffusion are the mechanisms controlling the release. The 3D-fibrillar network with 12-HSA as gelator is well documented in the literature and already observed in some of our previous works<sup>46,49</sup>. The driving force of controlled release is the entanglement of the network which represents a physical barrier to the diffusion of the entrapped oil containing the dissolved drug. Some interaction between EFV and 12-HSA could delay the free EFV diffusion through the 12-HSA network. However, it was not detected in this study by the Raman mapping of the organogel beads.

#### Conclusions

This study underlines the interest of gelling sunflower oil with 12-HSA for the controlled release of a hydrophobic drug like EFV. With organogels of different shapes and sizes, exposed to different release conditions, it could be demonstrated that the drug release was controlled mainly by diffusion with a minor participation of erosion in absence of enzymes, or controlled by more than one process, a coupling of diffusion and erosion mechanisms, the later accelerated by enzymatic activities.

Based on the drug release kinetics and mechanism models, EFV release from organogels could be described by the Korsmeyer-Peppas model with the highest coefficient of determination and lowest RMSE and AIC values. The release exponent ( $n$ ) allows highlighting the different EFV release mechanisms



with sunflower oil-based delivery systems: drug diffusion-controlled (sunflower oil,  $n=0.30$ ), anomalous transport (organogel beads,  $n=0.62$ ) and super Case-II transport (cylindrical organogels  $0.81 < n < 1.39$ ). Even though further data will be necessary to investigate EFV release kinetics in the 12-HSA sunflower oil gel more deeply, the obtained results have shown that it is possible to modify the drug release from the oil phase by gelling the system.

Furthermore, printing is a useful and simple technique to prepare organogel beads with spherical shape and narrow size distribution. The beads could be applied for the manufacture of multiparticulate dosage forms where a suitable controlled release rate could be designed to take advantage of beads properties. The multiparticulate nature of beads as a dosage form (filled into capsules) can offer important pharmacological and technological advantages over conventional single-unit solid dosage forms. Further optimization of formulation will be required before moving onto any *in vivo* studies.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This study was partially financially supported by the CNPq (Brazil).

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